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Impact of sucrose and boric acid on *in vitro* pollen germination of *Ceiba pentandra* L.

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Abstract

Studies on *in vitro* pollen germination and pollen tube length were carried out in *Ceiba pentandra* L. of the family Bombacaceae. Maximum 96% of pollen germination along with 4140±2211.48 µm pollen tube growth was recorded in 15% sucrose solution ($C_{12}H_{22}O_{11}$) supplemented with 100 µg/ml boric acid (H_3BO_3). Pollen abnormalities were recorded due to swelling and thickness of pollen tube and were recorded in sucrose solution but addition of boric acid facilitated an increased pollen germination as well as pollen tube elongation. Combine effect of sucrose and boric acid was significantly better than individual impact of sucrose alone on rate of *in vitro* pollen germination as well as pollen tube length. The pollen grains collected immediately after anther dehiscence (17:30-18:30 hrs.) showed best germination, but gradually decreased in course of time. The result revealed that sucrose acts as substrate for proper nutrition, while boric acid play active role on *in vitro* pollen germination as well as pollen tube elongation in the studied plant.

Key words: *Ceiba pentandra*, *In vitro* pollen germination, pollen tube.

Ceiba pentandra L. (Figure 1A) is commonly known as Kapok tree belonging to the family Bombacaceae with chiropterophilic flower[9] (Figure 1B). It is native to Asia and Africa and economically important due to commercial cotton yield. Beside its economical importance, the plant is also important for its medicinal aspects. Seed are edible and used as laxative, in leprosy and rheumatism[3]. The plant is night blooming with nocturnal anthesis that has chiropterophilic pollination[9&15]. The successful pollination is entirely on the mercy of bat and subsequent dissemination of viable pollen grains to the receptive part of the

stigma, because successful fertilization depends on the viability and fertility of the pollen grains. Receptive stigmatic surface is the ideal place for nourishment of the pollen grains, which in turn stimulate the pollen to germinate and guide the pollen tube to carry out the fertilization. But due to involvement of pistillate tissues in the stigmatic surface, it is difficult to carry out the biochemistry and physiology of pollen germination and pollen tube growth, which maximally can achieved through *in vitro* pollen germination studies. Due to its considerable medicinal as well as economical importance, the present study was carried out on *in vitro* pollen germination and pollen tube length for determination of pollen viability, fertility and pollen tube elongation before going to effective bereeding programme as pollen fertility and viability has a paramount importance in breeding programme.

In vitro pollen germination was conducted to determine the effect of sucrose ($C_{12}H_{22}O_{11}$) and boric acid (H_3BO_3) at various concentrations. For this purpose different grades of sucrose (1-40%) and boric acid (5-700 µg/ml) were prepared and used individually or in combination. During incubation, a dorp (50 µl) of each solution was poured into each groove of grooved slide individually or in combination and kept the slides in petridishes lined with moist blotting paper. Fresh pollen grains were collected just after anther dehiscence (17:30-18:30 hrs.) (Figure 1C) and were shown in nutrient medium and observed under Olympus microscope at stipulated period of time. All the experiments were performed in triplicate. Pollen grains were considered to be germinated when the pollen tube length was greater than the diameter of the pollen grains[4]. The results were taken and tabulated following the method of Shivanna and Rangaswamy[16] and analyzed using standard statistical method of Dutta[5].

In vitro pollen germination study showed 62% germinated pollen with a mean755±260 µm long pollen tube in 15% sucrose (Table-1). Individually boric acid did not show any role on *in vitro* pollen germination. But increasing pollen germination with increased pollen tube growth was observed in sucrose solution supplemented with boric acid. The best 96% pollen germination with a mean 4140± 2211.48 µm long pollen tube was resulted in 15% sucrose solution supplemented with 100 µg/ml boric acid after 12 hours of incubation (Table-2, Figure 1D & E). The statistical analysis indicate that the t-value is significant in between the pollen germination of G1 and G2 and pollen tube length of T1 and T2 at 0.01 level of probability respectively (Table-3), which suggested that combine effect of sucrose and boric is significantly better than individual impact of sucrose alone on rate of *in vitro* pollen germination and pollen tube growth of *Ceiba pentandra*. The result is attributed that sucrose acts as substrate for proper pollen nutrition and also functions as osmoregulator, while boron may enhance the sucrose uptake and stimulate germinating ability due to formation of a sugar-borate complex, which acts as better traslocator rather than non-borate, non-ionized sugar molecule[18]. Combine effects of sucrose and boric acid on increasing trends of pollen germination might be reflected the views of Johri and Vasil[7]. 30%

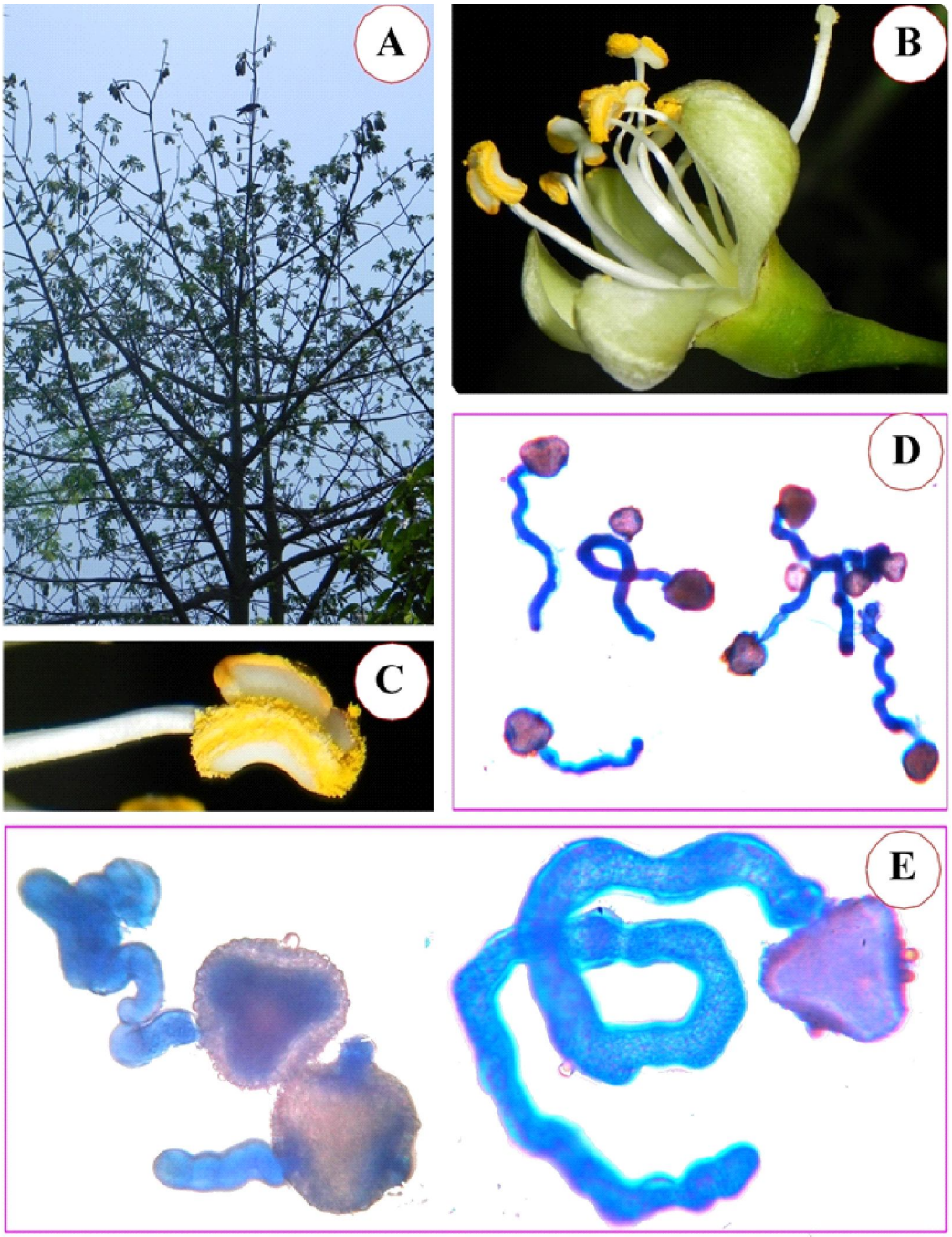

Figure1: A. Habit of plant, B. Night blooming flower, C. Dehisced anther with dusty pollen grains, D. Germinating pollen in low magnification and E. Germinating pollen with pollen tube.

Table-1. Effect of sucrose on *in vitro* pollen germination of *Ceiba pentandra*

Concen-tration(%)	After 4 hours		After 8 hours		After 12 hours	
	Germin-ation(%)	Tube length (µm)	Germin-ation (%)	Tube length (µm)	Germin-ation (%)	Tube length (µm)
Distilled water	-		-		-	
1	1	84±21.70 (Range 50-100, N=20)	4	180±75.27 (Range 50-250, N=20)	6	195±92.64 (Range 50-300, N=20)
3	4	125±58.92 (Range 50-200, N=20)	10	190±69.92 (Range 50-250, N=20)	15	205±64.33 (Range 100-300, N=20)
5	8	134±49 (Range 70-200, N=20)	15	222±103.58 (Range 100-400, N=20)	20	232±106.01 (Range 100-400, N=20)
7	12	287±123.02 (Range 70-500, N=20)	19	360±270.59 (Range 100-800, N=20)	25	365±240.88 (Range 100-850, N=20)
10	15	270±197 (Range 100-800, N=20)	28	415±176.46 (Range 150-650, N=20)	33	515±191.55 (Range 200-800, N=20)
12	20	315±108.14 (Range 100-500, N=20)	42	595±214 (Range 250-900, N=20)	46	610±243.58 (Range 300-900, N=20)
15	**33**	**322±119.05 (Range 120-500, N=20)**	**56**	**635±240.42 (Range 200-900, N=20)**	**62**	**755±260 (Range 400-1200, N=20)**
20	18	110±30.33 (Range 50-200, N=20)	30	340±112.54 (Range 150-500, N=20)	36	373±146 (Range 180-600, N=20)
25	8	73±14.94 (Range 50-100, N=20)	10	79±20.24 (Range 50-100, N=20)	15	92±26.99 (Range 50-150, N=20)
30	-	-	-	-	5	51±3.16 (Range 50-60, N=20)
40	-	-	-	-	-	-

± Standard Deviation, N= number of observation.

Table-2. Combine effect of sucrose and boric acid on *in vitro* pollen germination of *Ceiba pentandra*

Concentration (%+µg/ml)	After 4 hours		After 8 hours		After 12 hours	
	Germin-ation(%)	Tube length (µm)	Germin-ation (%)	Tube length (µm)	Germin-ation (%)	Tube length (µm)
15+5	22	590±185.29 (Range 400-900, N=20)	60	1140±471.87 (Range 300-1700, N=20)	65	1160±368.78 (Range 500-1800, N=20)
15+10	28	770±216.28 (Range 500-1200, N=20)	75	1452±924.44 (Range 900-4000, N=20)	78	1470±951.08 (Range 900-4000, N=20)
15+20	30	860±302.58 (Range 500-1500, N=20)	85	1550±969.82 (Range 500-4000, N=20)	88	1650±976.67 (Range 900-4000, N=20)
15+30	41	1000±368.17 (Range 600-1800, N=20)	89	1770±853.81 (Range 400-3500, N=20)	91	1810±917 (Range 900-4000, N=20)
15+40	46	1180±518.11 (Range 600-2200, N=20)	90	2160±1502.73 (Range 1100-6000, N=20)	92	2350±1543.62 (Range 1100-6000, N=20)
15+50	53	1580±859.97 (Range 800-3000, N=20)	93	3030±1241.90 (Range 1300-6000, N=20)	94	3040±1226 (Range 1400-6000, N=20)
15+100	**56**	**2290±1025 (Range 700-3500, N=20)**	**95**	**4070±3046 (Range 1800-10000, N=20)**	**96**	**4140±2211.48 (Range 1800-10000, N=20)**
15+200	46	1560±710 (Range 900-3000, N=20)	92	3310±1801.51 (Range 2000-7000, N=20)	93	3400±1429.84 (Range 2000-7000, N=20)
15+300	40	1030±510 (Range 400-2300, N=20)	88	2060±389.30 (Range 1700-3000, N=20)	90	2130±283.03 (Range 1700-2500, N=20)
15+400	31	930±444.84 (Range 400-2000, N=20)	75	1580±651 1110±578.21 (Range 700-3000, N=20)	78	1680±682.80 (Range 900-3000, N=20)
15+500	21	530±170 (Range 200-800, N=20)	68	1320±563.32 (Range 500-2500, N=20)	71	1470±621 (Range 500-2500, N=20)
15+600	15	490±144.91 (Range 300-800, N=20)	60	1250±533.85 (Range 700-2500, N=20)	63	1340±550.15 (Range 700-2500, N=20)
15+700	12	430±94.86 (Range 300-600, N=20)	50	1110±578.21 (Range 300-2200, N=20)	55	1180±482.58 (Range 300-2000, N=20)

± Standard Deviation, N= number of observation.

Table-3. Statistical analysis of percentage of pollen germination and pollen tube length at sucrose and sucrose supplemented with boric acid in *Ceiba pentandra*

Substrate	N	Mean	SD	SE	t-value	df	$P_{0.01}$	Significant at 0.01 level of probability
G1	10	26.300	18.147	5.738	7.9546	21	2.831	Significant
G2	13	81.076	13.732	3.808				
T1	10	339.300	229.346	72.525	6.4431			Significant
T2	13	2063.076	928.496	257.518				

G1- percentage of germination and T1-tube length in sucrose (15%); G2- percentage of germination and T2-tube length in sucrose (15%) supplemented with boric acid (5-700 µg/ml); N-number of observation; SD- Standard deviation in each group; SE- Standard error of difference of means; t-value-Student test value; df- Degree of freedom; $P_{0.01}$- Value of t for probability of 1% level.

pollen showed polysiphonous condition in sucrose solution. Polysiphonous condition became reduced when pollen grains were supplemented with boric acid. It has been suggested that boron may directly or indirectly influence the synthesis of callose and its subsequent distribution[6] and pectin for pollen tube elongation[10,17]. Such acidic pectin may enhance the tube strength by the accumulation of calcium[11,8]. The pollen grains collected immediately after anther dehiscence showed the best germination ability but gradually decreased with time and ultimately becomes nil during the second day of flower anthesis. The present findings are correlated with the findings of Vasil[19], Mondal *et al.*[12], Mondal *et al.*[13], Bhattacharya and Mandal[1], Biswas *et al.*[2], Mondal and Ghanta[14].

Thus viable pollen is prerequisite for successful pollination, which is an important tool for effective breeding programme. The knowledge of pollen viability chiefly depends on a number of biochemical sources and largely comes from *in vitro* germination study as it is possible to germinate pollen grains using different nutrients.

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